Journal of Ecobiotechnology 2/9: 14-20, 2010
ISSN 2077-0464
http://journal-ecobiotechnology.com/

Development of Quality Standards of *Corylus colurna* (Linn.) Fruit

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Keywords

C. colurna
Extractive values
Ash values

Abstract

Corylus colurna (Linn.) belonging to family Betulaceae is a deciduous tree distributed throughout the western temperate region of Himalayas in India. It is very valuable traditional herbal medicine. In present investigation an attempt has been made for the pharmacognostical standardization and evaluation of C. colurna fruit. The pharmacognostical evaluation comprises of detailed macroscopy, powdered microscopy, fluorescence analysis and physical constants such as ash and extractive values. The fruit extracts were subjected to preliminary phytochemical screening. The data obtained in present study will serve as valuable tool for identification, authentication and detection of adulterants, standardization and quality control of the drug. The developed technique will also be useful for the standardization of formulations containing *C. colurna*.

1. Introduction

Corylus colurna Linn. also known as Funduq (Urdu) and Turkish hazel (English) is distributed in western temperate Himalayas from Kashmir to Kumaon at the altitude of 1,700-3,300 m. This is extensively cultivated in Turkey for nuts and plants yield the fruits annually from fourth year onwards up to the 20th year [1].

The fruit possesses varied medicinal properties and therapeutic uses. It is used as a brain and intestinal tonic, aphrodisiac and expectorant and prescribed in weakness of brain and liver, gonorrhoea, and palpitation. It is mixed with honey and given as expectorant in cough and asthma [2,3]. Fruit also forms an ingredient of various compound Unani formulations like Majoon Ahrad Khurma, Laboob-e- Sagheer, Laboob-e-Kabeer, Halwa-e-Gazar, Majoon Falaksair and Roghan-e-Laboob-e-Saba [4].

Natural mixtures of flavonoids were isolated from the leaves of other species i.e. *C. avellane* L. Studied for strong antioxidant activity [5]. Besides, it was also a stronger inhibitor of ischaemia and reperfusion-induced peroxidation in the brain and liver.

Chemical composition of leaves and fruits in different species of *Corylus* including *C. colurna* in a particular period and the changes in the contents of carbohydrates, ten amino acids, carotene, rutin and some trace elements has also been reported [6].

C.colurna is a moderate sized deciduous tree and may attain height up to 17m. Leaves are petioled, glabrous, 12-18 cm long and 5-15 cm wide, having 10-12 pairs of nerves, terminating the lobes and are membranous. Male spikes are clustered, 2-5 cm long and very stout; bracts are obovate, acute with about 8 anther cells on the midrib and their filament variously connate. Nuts are globose and very hard. The tree bears nuts every third year and yields good harvest of nuts. It flowers before leafing; flowers appear in March-April and fruits ripen in the rains [1, 7, and 8].

Though, the fruits of the plant *Corylus colurna* Linn. (Trade name- Funduq) are used extensively as one of the ingredient of compound formulations in Ayurveda as well as in Unani system of medicine. There is no published work on standardization or pharmacognostical aspects of this drug. Literature survey clearly showed a thrust area to work on this drug.

2. Material and Methods

Chemicals and reagents

All the chemicals and reagents used were of analytical grade, purchased from Sigma chemical co. (St Louis, MQ, USA) and Merck (Darmstadt,

Germany). Fruits of *C. colurna* were collected from campus of Hamdard University (New Delhi) which was identified by Taxonomist (Professor M.P. Sharma), Department of Botany, Hamdard University New Delhi. The voucher specimen was deposited in Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard.

Morphological studies

The morphological studies were carried out for shape, size, colour, odour, taste and fracture of the *C. colurna* fruit.

Microscopic studies and powder analysis

The transverse section of fruit was prepared by standard method. Slides of powdered fruit material were also prepared and studied. Microphotography on different magnifications was carried out with motic microscopic unit. Polarized light was used for the study of crystals, starch granules and lignified cell.

Physicochemical standardization

The various physico-chemical values of fruit such as ash values, extractive values were determined according to the Pharmacopoeial method.

Fluorescence analysis

The fluorescence nature of powder drug was analyzed and the observations with different chemicals were also carried out and recorded.

3. Results and Discussion

Macroscopical evaluation

Market samples of fruits were dried nuts, hard, globose, almond colour, measuring and 1.3-1.7 cm in size. Longitudinal striations on the shell are present but at few places elongated depressions are also found almost in a significant manner. Seeds are globose with orange-brown seed coat possessing finger print like striations; kernel is cream coloured and much oily; fracture very difficult to break (woody); taste of the kernel is slightly sweet and pleasant but the odour is indistinct.

Dried nuts

Microscopical evaluation

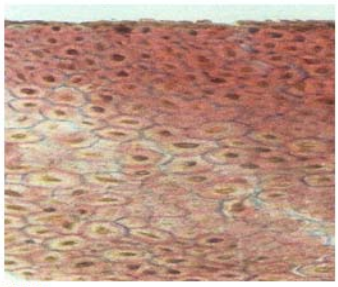
The transverse section of fruit is almost circular in outline. Epicarp is single layered and well cutinised; cells oval to sub-rectangular, measuring 9-18 x 6-9μ in size. Trichomes are also present on the epicarp that are simple, unicellular, thin-walled, and non-glandular and vary in size. Mesocarp is multilayered, generally composed of sclerieds, which are arranged in a very specific manner. The first few layers have smaller cells, middle portion possesses quite bigger cells while lower part has cells smaller than the upper portion; sclerieds are hexagonal to polygonal or oval, very thick walled with a broad lumen, having conspicuous and circular as well as vertical striations; measuring 9-76 x 6-36μ in size. Many vascular strands are present in this region. Vascular bundles prominently show the presence of vessels with associated parenchyma. Innermost part of the mesocarp is mainly consisted of collapsed cells.

Transverse section of the testa (seed coat) shows the outer part usually consisted of 2-3 layers of cells, which are simple, compact, rectangular, thin-walled parenchyma and measures 16-31 x 9-18μ in size. Some of these cells contain oils. Vascular strands are found only in the outer region, and surrounded by a layer of almost disorganized cells.

The cotyledon shows single layer of epidermis composed of oval to rectangular, slightly thick walled parenchymatous cells, which measure 13-22μ in length and 9-16μ in breadth. Rest part of the cotyledon is mostly consisted of compact, thin walled, hexagonal to polygonal parenchyma, measuring 18-50 x 11-36μ and mostly filled with oils. Small rosette crystals of calcium oxalate are also present in these cells.

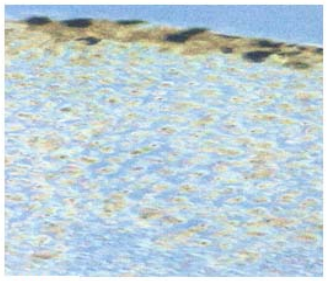
Single layered epidermis of radicle is usually made up of smaller, rectangular to squarish; thin walled parenchyma, measuring 13-22μ in length and 9-16μ in width. Rest part is consisted of hexagonal to polygonal, compact, thin walled parenchymatous cells, 13-27 x 12-18μ in size, and mostly filled with oils.

T.S of Testa



X 400

T.S of Cotyledon



X 400

Powder Analysis

Powder of the crude drug was brown, coarsed, free flowing. The taste was slightly sweet and pleasant but did not have any specific odour. Small amount of the powdered material (sieved through 40 mesh) was placed on the microscopic slide; mixed with a few drops of 40% w/v aqueous chloral hydrate and heated gently under Bunsen-burner. Few drops of 1% alcoholic phloroglucinol were added to this and warmed by mixing one drop of concentrated hydrochloric acid (HCl). The slides were mounted in glycerine and observed under microscope, which reveals the presence of fragments of epicarp, sclereids, testa, cotyledon, radicle and oil containing parenchyma. The stone cells or sclereids were abundant; hexagonal to polygonal, vary in size, highly thickened with broad lumen and conspicuous striations. Vessels were observed in lesser number, which were short, measuring 40-405 x 9-18μ thick walled having spiral or scalariform thickenings and their ends were almost rounded.

Maceration

Maceration of the fruit was carried out. The macerated tissues were strained in 1% saffranin in alcohol and mounted in glycerine to examine non-protoplasmic cellular contents, e.g. cells or fragment of fruit wall, mesocarpic scelreids, testa, cotyledon, and radicle. Oil containing parenchymatous cells was found in abundant but vessels were observed lesser in number. Details of the cells/ elements have already been discussed.

Plate-1

Fig .1 – T.S. of the fruit showing details of epicarp and mesocarp with the vascular region

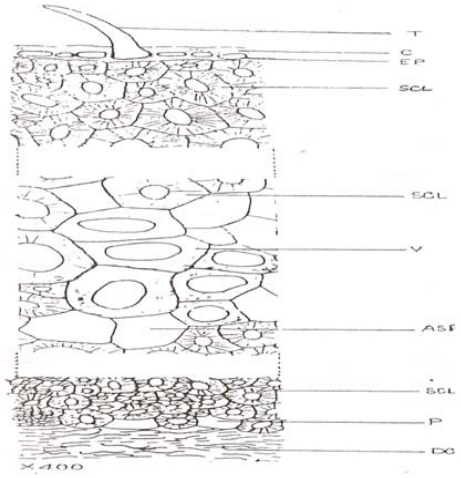


Plate-2

Fig. 2 – T.S. of testa through vascular supply

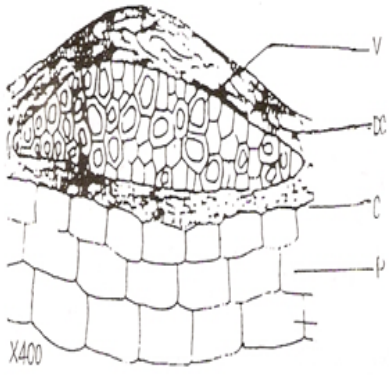


Fig. 3 – T.S of cotyledon

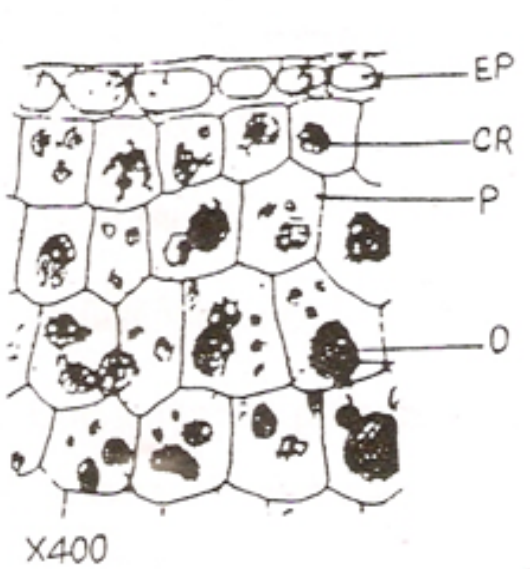


Fig. 4 – T.S. of radicle

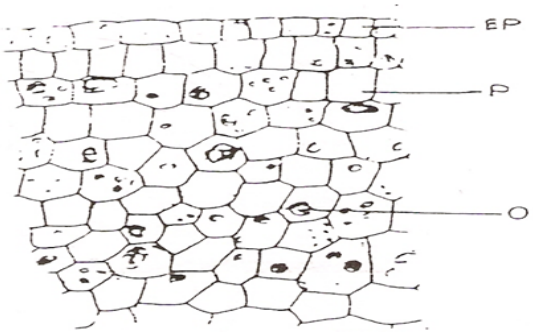


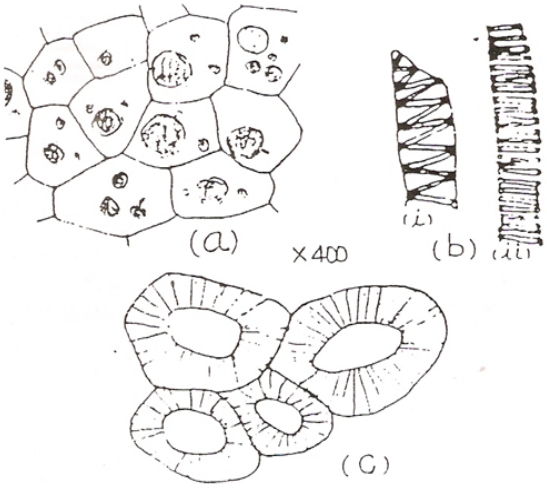
Fig. 5 – Surface view of:

(a) Cotyledonous parenchyma filled with oils, (b) Vessels -

(i) Spiral

(ii) Scalariform

(iii) Sclereids



Abbreviations:

ASP = Associated Parenchyma; C = Cuticle; CR = Calcium oxalate crystal; DC = Disorganized cells; EP = Epidermis; O = Oils; P = Parenchyma; T = Trichome; V = Vessel

Table-1 Reactions of Powdered Fruit of *C. colurna* with Different Chemical Reagents

S.NO.	Chemical Reagents	Observations
1.	Conc. Sulphuric acid	Reddish Black
2.	Conc. Hydrochloric acid	Dark brown
3.	Conc. Nitric acid	Orange
4.	Pot. hydroxide solution (aqueous) (5%)	Oily yellow
5.	Sodium hydroxide solution (aqueous) (5%)	Oily yellow
6.	Ferric chloride (aqueous)	Greenish blue
7.	Iodine solution	No change
8.	Picric acid	No change
9.	Acetic acid glacial	No change
10.	Powder as such	Brown

Table-2 Fluorescence Analysis of Powdered Fruit of *C. colurna*

S.No.	Reagents	Colour in Day Light	Observation Under U.V Light		
			Modifying Colour	Colour Quality	Degree of Radiance
	Mounted in Nitrocellulose	Brown	Brown	Light	Bright
2.	1N NaOH in MeOH	Brown	Pale green	Light	Bright
3.	Treated with 1N NaOH in MeOH and mounted in nitrocellulose	Reddish brown	Green	Light	Bright
4.	1N HCl	Orange	Reddish brown	Dark	Dull
5.	Treated with 1N HCl and mounted in nitrocellulose	Reddish brown	Green	Light	Bright
6.	1N NaOH in water	Dark brown	Pale green	Light	Bright
7.	Treated with 1N NaOH in water and mounted in nitrocellulose	Brown	Pale green	Light	Dull
8.	Diluted HNO_3 (1:1)	Orange	Brown	Dark	Dull
9.	Diluted H_2SO_4 (1:1)	Dark brown	Brown	Dark	Dull
10.	Powder as such	Brown	Reddish brown	Dark	Dull

Table-3 Ash Values of *C. colurna* Fruits

S.No.	Determinants	Values in Percentage
1.	Total ash	1.86
2.	Acid insoluble ash	1.42
3.	Water soluble ash	0.39

Table-4 Extractive Values of *C. colurna* Fruit

S.No.	Extractive Solvents	Values In Percentage *
1.	Petroleum ether (b.p. 60-80°)	6.52
2.	Benzene	0.89
3.	Chloroform	0.46
4.	Acetone	0.67
5.	Ethanol	0.76
6.	Distilled water	11.87

* Values are average of three determinations

Table-5 Preliminary Phytochemical Screening for Detection of Phytoconstituents from Ehtanolic Extract of *C.colurna* Fruit

S.No.	Phytoconstituents	Ethanolic Extract
1.	Acidic Compounds	-
2.	Alkaloids	-
3.	Carbohydrate	-
4.	Flavonoids	-
5.	Glycosides	+
6.	Phenolic compounds and tannins	+
7.	Proteins and free amino acids	+
8.	Resins	+
9.	Saponins	-
10.	Sterol and Triterpenoids	+

+ : Present - : Absent

4. Discussion

There is gradual revival of interest in the use and research of medicinal plants throughout the world, because herbal drugs are reported to be safe and free from side effects, which are generally associated with synthetics and antibiotics. Great emphasis is being given to explore the potential medicinal value of plants world over. The traditional systems of medicine are gaining recognition globally as more and more drugs are being chemically tested for ascertaining their therapeutic properties as mentioned in the texts. Many plants listed in Indian Pharmacopoeia are not being utilized by drug industries due to the lack of correct botanical identity, source(s), standardization and quality control of the plant material forming the drug. Now days the original drugs are either substituted or adulterated, whether willingly or unwillingly, it is very difficult to get a genuine sample of herbal drugs in the crude drug markets without special efforts. A plant material selected for research or medicinal purpose necessarily requires correct botanical source to achieve satisfactory results. Therefore, there is an essential need to have a comprehensive knowledge on the botanical principles associated with their description and evaluation.

Anatomical characters along with chemical features are of great value in the identification of plant drugs. Such studies will help not only in deciding the authenticity of drugs and the systematic position of the source taxon but also in detecting the adulterants.

The critical examination revealed that all the samples invariably contain the fruits of *C. colurna* Linn. Which is considered to be the source of plant of 'Funduq'. No adulterants have so far been reported for this drug but sometimes the fruits of allied species *C. avellana* Linn. Are also sold as 'Funduq' but the *C. avellana* can easily be distinguished on the basis of external characters, i.e. nuts are ovoid in shape and 1.0 to1.3 cm long in size.

The organoleptic, macro- and microscopy, preliminary phytochemical tests, fluorescence analysis, physical constant values and detailed phytochemistry have been studied in order to facilitate identification of genuine drug from any substitute of spurious sample. The relevant macro and microscopic features worth considering are:

1) Fruits are nuts globose in shape and 1.3-1.7 cm in size shows longitudinal striations on the shell and at some places elongated depressions are found in a very significant manner.

2) Seeds are globose with orange brown seed coat having finger like striations.

3) Thick walled parenchymatous epicarp.

4) Trichomes are simple, unicellular, thin walled, non-glandular and vary in size.

5) Multilayered mesocarp is consisted of sclerieds arranged in a very specific manner so that the middle portion possessed quite bigger sized cells and upper and lower portion have comparatively smaller cells.

6) Sclerieds are hexagonal to polygonal or oval, very thick walled with a broad lumen, having conspicuous and circular as well as vertical striations.

7) Innermost layer of mesocarp is composed of collapsed cells.

8) In testa, vascular strands are found only in the outer side and surrounded by a layer of almost disorganized cells.

9) Parenchyma of cotyledon has smaller rosette crystals of calcium oxalate.

10) Vessels have spiral or scalariform thickenings.

Powder of the drug, sieved through 40 mesh, was studied microscopically and revealed the presence of fragments of epicarp, sclereids, testa, cotyledon, radicle and oil containing parenchyma. The stone cells or sclereids were abundant, hexagonal to polygonal; vary in size, highly thickened with broad lumen and conspicuous striations. Vessels were lesser in number and with spiral or scalariform thickenings. Macerate revealed the presence of fruit wall; mesocarpic sclereids, testa, cotyledon, radicle and oil containing parenchymatous cells in abundant but vessels were observed in lesser number. Fluorescence analysis of powder of *C. colurna* after the treatment with different reagents and observed under ordinary light then under ultraviolet light showed different colour as shown in **Table-2**. But powder with 1N NaOH in methanol and 1N NaOH in water showed a remarkable colour, i.e. bright light pale green under ultraviolet light. The microchemical and preliminary phytochemical tests showed the presence of phenolic compounds, tannins, glycosides, proteins and free amino acids, resins and sterol and triterpenoids. The total ash, acid insoluble ash and water soluble ash values were determined to be 1.86%, 1.42% and 0.39% respectively. The extractive values of *C. colurna* in different solvents were determined as an average of three determinations. The maximum extractive value (11.87%) was found in distilled water and minimum (0.46%) in chloroform and in other solvents like petroleum ether (60-80°) - (6.52%), benzene (0.89%), acetone (0.67%) and ethanol (0.76%).

Reference

1. Anonymous (1950). *Wealth of India*, Vol. II, PID, CSIR, Hillside Road, New Delhi, 358.
2. Kirtikar, K.R. and Basu, B.D. (1975). *Indian Medicinal Plants, Vol.III,* L.M. Basu, Allahabad, 2359.
3. Chopra, R.N. ; Nayar,S.L. and Chopra, I.C. (1956). *Glossary of Indian Medicinal Plants*, PID, CSIR, Hillside Road, New Delhi, 78.
4. Anonymous (1982). *National Formulary of Unani Medicine*, Ministry of Health and Family Welfare, Govt. of India publication.
5. Benov, L and Georgiev, N (1994). The antioxidant activity of Flavonoids isolated from *Corylus colurna, Phytotherapy Research* 8 (2): 92-94.
6. Ronkov, B.; Staeva, L; Lachkova, V and Evangelatova, N (1983). Chemical composition of the leaves and fruits of different species of *Corylus. Nauchni-Trudove-Lesote-Khnicheski-Institut, - Sofiya, -Gorsko-Stopanstvo No.*27-28, 133-141.
7. Anonymous (1994). *Dictionary of Flowering Plants in India*, PID, CSIR, Hillside Road, New Delhi.
8. Hooker, J.D. (1973). *Flora of British India*, Periodical Experts, New Delhi, 625.